October 6, 2000

Kathleen Roberts
Manager, Petroleum Additives Panel Health,
Environmental, and Regulatory Task Group (HERTG)
American Chemistry Council
1300 Wilson Blvd.
Arlington, VA 22209

Dear Ms. Roberts:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Alkyl Sulfides category, submitted March 28 and April 24, 2000. I commend the HERTG for their commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will adequately characterize each SIDS endpoint. On its Chemical RTK HPV Challenge Program website EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the attached Comments on the Chemical RTK web site within the next few days. As noted in the comments, we ask that the Panel advise the Agency, within 60 days of the posting on the Chemical RTK website, how it intends to pursue its activities on these chemicals.]] Please respond either by email (oppt.ncic@epa.gov, hpv.crtk@epa.gov, or chem.rtk@epa.gov)or by regular mail to:

Carol Browner, Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Attention: Chemical Right-to-Know Program

EPA will post your response on the Chemical RTK website.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-260-3470. Submit general questions about the HPV Challenge Program through the Chemical RTK web site comment button or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@.epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

/s/

Oscar Hernandez, Director
Risk Assessment Division

Attachment

cc: W. Sanders
C. Auer
N. Patel
A. Abramson

EPA Comments on Chemical RTK Challenge Submission: Alkyl Sulfides

SUMMARY OF EPA COMMENTS

The sponsor, the CMA (now ACC) Petroleum Additives Panel's Health, Environmental, and Regulatory Task Group (HERTG), submitted Robust Summaries and a Test Plan and Category Justification to EPA dated March 28, 2000. EPA posted the submission on the ChemRTK Web site on June 13, 2000. The proposed information-gathering plan is for five substances: 1-Dodecylthio-2-propanol (CAS No. 67124-09-8); Sulfurized 1-decene ("decene derivative", CAS No. 72162-15-3); Sulfurized 2-methyl-1-propene ("methyl propene derivative", CAS No. 68511-50-2); Sulfurized 2,4,4-trimethylpentene ("trimethyl pentane derivative", CAS No. 68515-88-8); and Sulfurized C15-18 alpha-alkenes ("C15-C18 alkene derivative", CAS No. 67762-55-4), all considered by the sponsor to constitute an Alkyl Sulfides category.

EPA has reviewed this submission and has reached the following conclusions (more detailed discussions appear in the main text):

1. For four of the chemicals, the submission comprised a minimally acceptable category submission for some endpoints. While the category argument was supportable for ecotoxicity and environmental fate, data were insufficient to support the category approach as proposed for health effects.

2. The justification for including 1-dodecylthio-2-propanol (CAS No. 67124-09-8) as a category member for health endpoints is weak, given the differences in its structure compared to other members. Some of the reasoning in the test plan for its inclusion appears contradictory.

3. Chemical characterization. Percentage composition of mixture constituents would be helpful in examining test substance selection for ecotoxicity and health effects. In particular, it is important to know the relative amounts of lower molecular weight components (below MW 500), as these are more likely to contribute to toxicity.

4. Physicochemical properties and environmental fate:

(a) Certain measured values should be supplied. In particular, boiling points (decomposition points if appropriate), water solubilities, and vapor pressures are needed unless precluded by experimental obstacles. The use of estimated values introduces uncertainties that then become magnified in modeling applications. Water solubility measurement is also important because it can help determine the need for aquatic toxicity testing of more hydrophobic chemicals.

(b) If the nature of the substances makes experimental determination of some or all of the usual physical properties impractical, the sponsor should consider what other measured properties might be supplied in order to characterize the chemicals and establish a baseline for evaluation of the category members.

(c) The selection of compounds to represent some category members for modeling purposes should cover more of each substance's range of structures.

(d) EPA agrees that fugacity-based modeling can be used to estimate transport and distribution of these chemicals. However, EPA recommends that the EQC Level III model be used to estimate transport and distribution rather than the Level I and II models because it provides a more sophisticated level of analysis.

(e) EPA agrees with the modeling approach to atmospheric photodegradation. However, photolysis can also occur as a result of absorption of solar radiation. The disulfide bonds in some of the compounds in this category can absorb UV light at a wavelength that could result in cleavage of those bonds. Therefore, direct photolysis studies of these substances are also appropriate.

5. Health effects.

(a) The selection of CAS No. 67124-09-8 for health effects testing as the potentially most toxic substance and the feasibility of basing a category analysis on these data were not adequately substantiated.

(b) Available health effects data represent studies performed by oral, dermal, and inhalation routes. A discussion of the most appropriate exposure route for these substances vis-a-vis planned testing would

be useful.

(c) EPA questions the proposal that a one-generation study (OECD 415) would satisfy the SIDS developmental toxicity endpoint.

(d) The proposed test plan is to test only CAS No. 67124-09-8, and no arguments are presented as to why the results would be relevant to the other four members of the category.

6. Ecological Effects.

(a) EPA believes that the reported aquatic toxicity studies on CAS No. 68511-50-2 are inadequate because they were not performed according to the most appropriate procedures for hydrophobic chemicals. Aquatic toxicity studies of substances such as the alkyl sulfides that have low water solubility should be performed at or below their water solubility limit.

(b) EPA further believes that such substances are not likely to pose acute aquatic hazards, but may have the potential to pose chronic aquatic hazards, and that the 96-h fish and 48-h daphnid acute tests are too short in exposure duration for hydrophobic compounds of this type. Instead EPA suggests that the daphnid chronic flow-through test be done for the representative chemicals. The 21-day chronic daphnid test, unlike the acute tests, is more likely to detect toxic effects at or below the aqueous solubility limit, owing to the longer exposure period for the chemical to reach equilibrium between the water phase and the test organism.

(c) Chronic aquatic testing may not be necessary if the water solubilities are sufficiently low. EPA suggests that measured water solubility tests for these substances will clarify the need for chronic toxicity testing.

(d) EPA agrees with the sponsor that aquatic toxicity testing on at least two category members is necessary to characterize the category for this endpoint, unless contraindicated by measured water solubility data. Although it is reasonable to test CAS No. 67124-09-8 as the least hydrophobic member, it is not clear which of the remaining chemicals is the preferred second candidate. EPA estimates that even the most water-soluble components of CAS No. 68515-88-8 and CAS No. 67762-55-4 are likely to be too hydrophobic to show aquatic toxicity, in contrast to the other three members. EPA suggests that measured water solubility data for these substances can help identify the best candidate from CAS Nos. 72162-15-3 and 68511-50-2.

7. As with all category proposals, the outcome of the proposed testing may change the approach or viability of the category compared to the original proposal.

EPA is requesting that the sponsor advise the Agency within 60 days how it intends to pursue activities on the chemicals in its submission.

EPA COMMENTS ON THE ALKYL SULFIDES CHALLENGE SUBMISSION

GENERAL

The sponsor supplied a complete package. The test plan addressed all endpoints and the robust summaries were reasonably well organized.

The sponsor provided the range of possible structures for each CAS number. However, the ranges of possible percent composition for the different compounds within the mixtures, even in an approximate way, were not given. This makes it difficult to evaluate the proposal for some endpoints, with respect to choice of test substance, for example, because of the wide molecular weight variation within some mixtures and because these ranges can include structures of greater and lesser potential concern within a mixture. EPA suggests that the percentage composition for each mixture component be provided in each appropriate robust summary (under “Test Substance Remarks”) in order to more fully characterize the test substance used in the study described.

In the case of mixtures it is also useful to know something about the analytical and other methods used to characterize the substances; this makes it easier for reviewers to comment usefully on aspects of the proposal with less need to speculate about substance characterization, ability to measure concentrations, etc.

CATEGORY DESCRIPTION

These chemicals are all of relatively high molecular weight (>250) with similar physical characteristics. The stated lubricant additive use demands that the chemicals share certain properties, i.e., hydrophobic, lipid-like substances with low volatility and chemical reactivity.

The sponsor proposes that the five named substances constitute a category based on "...structural similarities and limited reactivity, low biological activity, and very low water solubility". No experimental data are supplied to confirm this and only estimated values of various properties are presented. The situation is highly complex in that the five substances named in the proposal constitute one discrete chemical and four mixtures; two mixtures contain both linear and cyclic materials; some substances are linear and others highly branched; and molecular weight ranges vary. The molecular weight range of 320–2,300 for CAS No. 68511-50-2 is broad relative to the other proposed category members.

Some aspects of the proposal raise questions about the sponsor's intent. For example, some compounds chosen for modeling purposes seem outside the range of indicated possible structures, leaving it unclear whether the substance definition or the model compound is correct. This problem is addressed in more detail below under "Test Plan".

CATEGORY JUSTIFICATION

The sponsor presents a case for considering the named alkyl sulfides as a category. EPA believes the presentation offers support for this proposal to evaluate environmental fate and effects. However, for health effects endpoints, the case is less solid. The sponsor states that the five chemicals should be treated as a category and that 1-dodecylthio-2-propanol, CAS No. 67124-09-8, is presumed to be the most toxic member of the category. Therefore, the submitter proposes testing with that compound only (for reproductive/developmental toxicity - the only health endpoint for which there are no data) and have the results apply to the other four category members. There are several reasons why this may not be appropriate:

1. The proposal that CAS No. 67124-09-8 has the highest toxicity potential is stated as fact without support. While this position may be arguable, some countervailing factors are not mentioned, such as the difference in reactivity between sulfide and disulfide groups: disulfides can be cleaved to monosulfur derivatives in biological systems, while monosulfides cannot undergo this reaction Although this distinction may not be relevant for most of these rather high molecular weight substances, it should not be implied that sulfur compounds are biologically inert.

2. The discussion of the potential metabolism and conjugation of CAS No. 67124-09-8 is hard to follow, with the discussion of potential detoxification appearing to conflict with the statement immediately following that "Consequently, this substance is expected to share the same toxicological properties as the rest of the category." The potential metabolism of this compound is unique among the category members and suggests that this substance should not be included in the category for health endpoints. For similar reasons, the proposal that data on this substance alone would be sufficient to characterize certain health effects is not persuasive; test results from this chemical may not be applicable to the other four.

3. The acute toxicity and genotoxicity data do not show any difference (qualitative or quantitative) between CAS No. 67124-09-8 and the other test materials. The available data on acute toxicity and genotoxicity show low acute (lethality) toxicity and negative results, respectively, for the four chemicals tested; the only one not tested for either endpoint was CAS No. 72162-15-3. Thus, *for the acute toxicity and genotoxicity endpoints*, the biological responses appear too low to rank the substances for biological activity within the proposed category.

4. The repeat dose data presented, although difficult to interpret in a category context because each CAS number was tested by a different route of administration, do suggest there may be differences in toxicity among the substances tested. Details are provided in the section on "Specific Comments on Robust Summaries".

Thus, EPA questions the appropriateness of the category approach for the repeat dose and reproductive/developmental endpoints (see below under Test Plan for more comments).

TEST PLAN

General

As presented in the robust summaries for all endpoints, the chemicals identified by the sponsor as representative of the five substances are inadequately described or may not adequately represent the mixtures, and the test plan does not fully justify their selection. For example, one of the chemicals selected for modeling as representative of CAS No. 72162-15-3 is a monosulfide (x = 1, MW=314.6; see Robust Summary, Application of Environmental Fate Modeling, Tables 1-3). This chemical, however, is not included in the group as presented in Figure 1 of the submission, as Figure 1 indicates that only di- and trisulfides (x = 2–3) are present in CAS No. 72162-15-3. It also appears that the two chemicals modeled for this substance are the non-cyclic forms, but this is not explicitly stated and no rationale was presented. Other examples are presented below under "Chemistry." The cyclic compounds may behave differently than the acyclic compounds and, in the absence of justification for modeling only one type, representatives from both groups should be considered.

Chemistry (melting point, boiling point, vapor pressure, water solubility, and partition coefficient).

Certain measured values should be supplied for these substances. In particular, boiling points (decomposition points if appropriate), water solubilities, and vapor pressures are needed unless precluded by experimental obstacles. The use of estimated values introduces uncertainties that then become magnified in modeling applications. If the nature of the substances causes unsurmountable difficulties in measuring the usual physical properties, the sponsor should consider what other measured properties might be supplied that can characterize the chemicals and establish a baseline for evaluation of the category members.

The sponsor calculated data on individual mixture components, using EPIWIN, to provide presumably representative or typical values. For mixtures it seems useful to calculate values for both low and high molecular weight components to provide ranges of values for the different mixtures.

For CAS No. 68511-50-2, it appears that the chemicals modeled are both monosulfides (x = 1), although this is not stated. CAS No. 68511-50-2 as described in Figure 1 includes mono through pentasulfides (x = 1–5); therefore a higher sulfide should also be considered for modeling. For CAS No. 68515-88-8, the first chemical modeled apparently is a monosulfide (x = 1, y = 1), although the substance according to Figure 1 only includes tetra- and pentasulfides (x = 4–5). Neither of the entries in Tables 1–3 representing CAS No. 68515-88-8 specifies the value of x, and both are outside the molecular weight range specified in Figure 1. In addition, unlike the other mixtures, no molecular weight range is provided for the Figure 1 structure representing CAS No. 67762-55-4. Thus, it is unclear why some of the structures chosen were considered to be representative of a given mixture.

Finally, as described below in the ecotoxicity section, it appears that water solubility studies would help understand the potential for aquatic toxicity of the potential test substances.

Fate (photodegradation, stability in water, biodegradation, and transport/distribution).

EPA agrees with the environmental fate sections of the proposed Test Plan with the following exceptions as to transport/distribution and photodegradation.

EPA agrees that fugacity-based modeling can be used to estimate transport and distribution of these compounds. However, EPA recommends that the EQC Level III model (available free from http://www.trentu.ca/academic/aminss/envmodel) be used to estimate transport and distribution rather than the Level I and II models because it provides a more sophisticated level of analysis.

The Test Plan states that "EQC Level III was not conducted for the present evaluation since the physical properties of the chemicals will not result in emissions or transport to air or water." However, use of the EQC Level III model for 1-dodecylthio-2-propanol, CAS No. 67124-09-8, which is the most water soluble member (0.307 mg/L; EPI Program, WSKow v1.37) and one of the lowest molecular weight (MW =260) species of this category, yields a distribution (%) of air (0.25), water (10.9), soil (39.4) and sediment (49.5). The Level I model (Table 2 in the Robust Summary) yielded air (0.3), water (0.4), soil (97.1) and sediment (2.2). Hence the EQC Level III model indicates a significantly different distribution, e.g., in water, 10.9% vs. 0.4% from the EQC Level I model. The two models produce similarly divergent results for other substances in the category: for the much less soluble decene derivative (CAS No. 72162-15-3),

where x=2, EQC Level III predicts 7.3 % to water vs. 0.000003 % via Level I.

In the photodegradation section (Section 3.1.4, p.7), the sponsor dismisses the potential for direct photodegradation. Photodegradation can occur directly or indirectly. Indirect photolysis can occur in the atmosphere as a gas phase oxidation reaction by photochemically produced oxidants such as hydroxyl radicals, which the sponsor states can be evaluated with the AOPWIN modeling program. EPA agrees with this part of the test plan. However, photolysis can also occur as a result of absorption of solar radiation. Except for CAS No. 67124-09-8, all category members include polysulfide bonds. Disulfide bonds can absorb UV light at a wavelength of about 365 nm and possibly result in cleavage of these bonds. Possible adsorption to soil particles does not preclude these types of compounds from undergoing photolysis. By concentrating on indirect photolysis, the sponsor may have underestimated the rate of degradation of these chemicals. This uncertainty can be addressed by conducting direct photolysis studies. EPA is aware that there are no OECD test guidelines for direct photolysis; however, EPA has such test guidelines available (see guidelines 835.2210 and 835.2310 at http://www.epa.gov/docs/OPPTS_Harmonized/).

Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

EPA has the following comments on the proposed health effects portion of the test plan:

5. Use of CAS No. 67124-09-8, 1-dodecylthio-2-propanol, as the most toxic category member. EPA disagrees with the proposed test plan to evaluate only CAS No. 67124-09-8 for the reproductive/developmental endpoint and have that result apply to the four other chemicals proposed in the category. EPA is not convinced that a category approach is appropriate for this endpoint, given the repeat-dose study results presented (see specific comments on robust summaries).

6. Reproductive/developmental toxicity. EPA also would like to point out that the proposed test protocol (a one-generation study, OECD Test Guideline 415 as cited on page 21 of the test plan) is not acceptable for both reproductive and developmental endpoints under the OECD SIDS program. An alternative is to conduct the combined reproductive/developmental test protocol, or OECD 421.

7. Repeat dose toxicity. EPA is not convinced that a category approach is appropriate for the repeat dose endpoint, given the repeat-dose study results presented. This is because no consistent observations were obtained when different CAS numbers were tested. These points are described in more detail in a subsequent section.

8. Route of exposure. EPA is concerned about the route of exposure that would be used in whatever tests are performed. Based on the use of these materials (lubricant additives), and the data that have been collected to date, the most appropriate routes of exposure in terms of relevance to humans appear to be either the inhalation or dermal routes.

Ecological Effects (fish acute, invertebrate acute, and algal acute toxicity).

EPA has the following comments on the proposed ecological effects portion of the test plan:

2. The category justification for the alkyl sulfides appears viable and consistent with an understanding that the high hydrophobicity of these chemicals correlates with low acute aquatic toxicity potential.

2. EPA further believes that such substances do not likely pose acute aquatic hazards, but may have the potential to pose chronic aquatic hazards. In EPA's experience, for certain chemicals exerting narcosis-based effects a measured water solubility value can be used to determine if chronic testing is appropriate. Therefore, EPA suggests that measured water solubility data for potential test substances will clarify this point.

3. EPA agrees with the sponsor that aquatic toxicity testing on at least two category members is necessary to characterize the category for this endpoint, unless contraindicated by measured water solubility data. With respect to the choice of test substances, EPA would prefer that the least hydrophobic chemicals be tested to satisfy the data set. While CAS No. 67124-09-8 is probably the most soluble/least hydrophobic category member, it is uncertain which is the least

hydrophobic of the remaining members. A preliminary EPA analysis suggests that even the most water-soluble components of CAS No. 68515-88-8 and CAS No. 67762-55-4 are likely to be too hydrophobic to show aquatic toxicity, in contrast to the other three members. Thus the best additional candidate for this testing is likely to be either CAS No. 72162-15-3 or CAS No. 68511-50-2. Measured water solubilities should clarify this question.

4. Fish acute toxicity: Daphnid chronic study as alternative. The proposed 96-h acute test on 1-(*tert*-dodecylthio)-2-propanol (CAS No. 67124-09-8). The 96-h acute test is too short in exposure duration for hydrophobic compounds of this type. Instead EPA suggests the daphnid chronic test (OECD 211) using a flow-through system (contingent on water solubility measurement). The 21-day chronic daphnid test, unlike the acute tests, is more likely to detect effects at or below the aqueous solubility limit owing to the longer exposure period for the chemical to reach equilibrium between the water phase and the test organism.

   The Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000 - available on the OECD Web site at http://www.oecd.org/ehs/test/monos.htm) states that the test concentrations should be calculated from the measured concentrations of test substance and should be within the test substance water solubility range. In addition, reasonable efforts should be made to measure the water saturation concentrations for this chemical or to report a preliminary experiment demonstrating that the test sample preparation regime is sufficient to maximize the concentration of the test substance. While the guidance discusses the use of water-accommodated fractions, it also states that test designers should refer to the regulatory requirements of individual sponsor countries in order to provide the most appropriate test method for determining the toxicity of low-water-solubility chemicals.

5. Daphnid acute toxicity. EPA recommends forgoing the daphnid acute toxicity testing in favor of the daphnid chronic test mentioned above. The proposed daphnid acute test is too short in duration for the chemical to reach equilibrium with the organism and therefore probably will not provide meaningful results.

6. Algal toxicity. EPA agrees with the proposal to conduct a 96-h algal EC50 toxicity test on 1-(*tert*-dodecylthio)-2-propanol, but also suggests that the same test be done with CAS No. 68511-50-2 (or other substance as determined by measured water solubility). Toxicity to algae may be observed in hydrophobic chemicals of this type in the 96-h exposure duration owing to their ability to go through six to eight generations (depending on species) and is considered a chronic test. The test should be conducted to determine biomass (cell density/mL) at or below the water solubility limit of the chemical using measured concentrations. The test should be conducted in a manner prescribed by the difficult substances and mixtures guidance (cited above) with regard to proper test substance preparation to determine the water solubility limit. Measured concentration values should be provided for the NOEC and LOEC.

SPECIFIC COMMENTS ON ROBUST SUMMARIES

EPA evaluations are based on the guidance document available at http:\\www.epa.gov/opptintr/chemrtk/guidocs.htm.

Chemistry

All of the physical properties presented in the review were estimated by the EPI program. As noted above, measured values should be supplied for boiling points, water solubilities, and vapor pressures unless precluded by experimental considerations. Furthermore, the estimates in the alkyl sulfide category review were performed on single structures, while four of the five category members are mixtures. This makes the melting point and boiling point estimations more difficult to predict. The tables of values clearly state that these values are estimates from modeling programs and state which chemical structure was run through the program. According to the sentence beginning on the last line of the first page of the summary titled “Application of Environmental Fate Modeling to HERTG HPV Group 1 - Alkyl Sulfides Category”, “...basic physical properties of over a dozen structures were estimated using EPIWIN and 9 of these are shown in Table 1". There is little rationale stated for choosing either the 12 or 9 substances named.

Environmental Fate

Two biodegradability robust summaries were submitted. The sponsor's Robust Summary treatment of this endpoint is sufficient, except that the summary for CAS No. 68511-50-2 does not characterize the percentage composition of the test substance.

EPA has some comments on the EPIWIN summary:

3. The sponsor omitted a factor of 10^{-12} in the atmospheric oxidation rate for all values in that column in Table 1.

4. As noted elsewhere, there is no rationale or justification presented for only modeling the linear or branched compounds rather than the cyclic components of the substances.

Health Effects

EPA evaluated each acute, repeat dose, and genotoxicity robust summary (26 summaries in all). Most of the summaries were considered adequate for the purposes of the U.S. HPV Challenge Program. In some cases, inadequacies were identified, primarily in some genotoxicity summaries, but they were not considered critical enough to offset the overall negative results obtained for that endpoint. In these cases, it would be useful if the comments raised could be clarified by examining the full study report.

Acute Toxicity. Eleven robust summaries describing acute toxicity studies were submitted involving four of the five alkyl sulfide category members (the exception being CAS No. 72162-15-3). All eleven summaries were considered adequate for the purposes of the U.S. HPV Challenge Program. EPA has the following comments on the summaries:

CAS No. 67124-09-8 (1-dodecylthio-2-propanol)

Two GLP studies were submitted, one with rats (oral, gavage) and one with rabbits (dermal). In both robust summaries, the same typographical error was made: the units for the limit dose in the "Remarks field for test conditions" section should be in grams, not milligrams.

CAS No. 68511-50-2 (methyl propene derivative)

Three studies were submitted, two non-GLP oral (gavage) rat studies and one GLP inhalation rat study. EPA has no comments on these summaries.

CAS No. 68515-88-8 (trimethyl pentene derivative)

Five GLP studies were submitted: three inhalation studies (rats, mice and guinea pigs); one oral (gavage) rat study; and one dermal study (rabbit).

In the oral and dermal robust summaries, a typographical error was made in presenting the units for the limit dose in the "Remarks field for test conditions" section: they should be grams, not milligrams.

CAS No. 67762-55-4 (C15-C18 alkene derivative)

One GLP study was submitted on this chemical: a dermal study with rabbits. EPA has no comments on this study.

The existing data show that the four tested compounds are similar in that they have low acute toxicity by a variety of routes (oral, dermal and inhalation) and there were no remarkable gross pathological findings in surviving animals. From the information presented in the summaries, two apparent differences among the four are: (1) oral and ocular discharges were observed in oral and dermal studies with CAS No. 67124-09-8 and the inhalation study with CAS No. 68511-50-2, and (2) an apparent sex difference was observed in rats exposed to CAS No. 68515-55-4 via inhalation (females more susceptible than males) - this was not observed with any other route/species for this compound. Finally, more information on the percentage composition of the mixture components in the three methyl propene derivative studies would

be helpful.

From the perspective of the proposed category, these data suggest that responses appear too low to rank the substances for biological activity. With such responses it is hard to draw conclusions about the category other than confirmation of the expected low activity. The data provide no evidence to confirm the proposal that CAS No. 67124-09-8 is the most toxic member.

Repeat Dose Toxicity. Six robust summaries describing repeat dose studies were reviewed, involving three of the five alkyl sulfide category members (the two not tested were CAS Nos. 72162-15-3 and 67762-55-4). All of the summaries were considered adequate for the purposes of the U.S. HPV Challenge Program. Following are EPA's comments:

CAS No. 67124-09-8 (dodecylthio-2-propanol)

> The summary describes a 28-day oral study (run under GLP conditions) in rats at doses of 0, 100, 300, and 1000 mg/kg which was followed by a 14-day recovery period for the control and high dose groups only. There was no NOAEL observed in this study. Effects observed (all resolved in recovery group unless otherwise noted): males and females–dose-related elevations in liver weights and liver/body weight ratios, hepatocyte hypertrophy (not resolved); males only–increased kidney weights, kidney/body weight ratios, pale or tan kidneys, existence of globular casts and hyaline droplets.
>
> While EPA agrees with the sponsor that the male kidney effects may be related to alpha$_{2\mu}$-globulin, the date of the study (1991) suggests that immunochemical identification of the alpha$_{2\mu}$-globulin protein should have been done to verify this hypothesis (this may have been done but not noted in the robust summary). Finally, because microscopic liver effects persisted during the two-week recovery period, EPA does not agree with the submitter that the hepatic effects are likely adaptive in nature and thus not a toxic effect.

CAS No. 68511-50-2 (methyl pentene derivative)

> Three dermal studies were submitted, one 90-day rat study and two rabbit studies (one 28 days and one 21 days). The rat and 28-day studies were run under GLP conditions whereas the 21-day dermal study was not.
>
> In the rat study (six doses from 10 to 2000 mg/kg), results showed a variety of effects (primarily at doses of 250 mg/kg and higher): males only–reduced body weight gain, increased kidney weights and hyaline droplet formation; both sexes–changes in hematological parameters, increased spleen size and liver/body weight ratios, and moderate to strong skin reaction. A NOAEL of 50 mg/kg was identified for increased production of white blood cells in the spleen and bone marrow of both sexes.
>
> In the GLP rabbit study (28 days, 200 or 2000 mg/kg), results showed a trend of weight loss and food consumption in high dose males (one male died). Severe skin irritation and some hematological effects were observed at both doses (no NOAEL was noted).
>
> In the non-GLP rabbit study (21 days, 140, 560, or 2440 mg/kg), results showed severe erythema with cracked/bleeding skin, eschar and discoloration and slight to moderate edema. Epithelial hyperplasia was observed in all rabbits (but more in treated groups). No NOAEL was noted.

CAS No. 68515-88-8 (trimethyl pentene derivative)

> Two GLP studies were submitted on this chemical: one dermal (rat, males only, 28-days) and one inhalation study (rats, 28-days).
>
> In the dermal study, male rats were exposed to 0 or 1000 mg/kg test material and the only effect observed was weak to moderate irritation (erythema, eschar formation and flaking of skin over the course of the study).
>
> In the inhalation study, rats were exposed to 0, 15, 50, or 150 mg/m^3 (equivalent to 0.015, 0.05, or 0.15 mg/L) test material for 6 hrs/day, 5 days/wk for 4 wks with a 3-week recovery period for control and high dose animals. Effects observed that did not recover over the three week

recovery period were: (both sexes)–decreased body weight gain, increased spleen and adrenal weights (no mention if recovered) and post-recovery increase in testes, lung, and heart weights; males–histopathological kidney effects (globular casts at the corticomedulary junction, cortex, and medulla and hyaline droplets in proximal convoluted tubules).

Taken together, the results of the six studies on the three chemicals suggest that all three chemicals have the potential, via the oral, dermal or inhalation routes, to induce hyaline droplet formation in male rat kidneys which may be associated with the alpha$_{2\mu}$- globulin protein. However, using the data in a category context suggests that the category may not hold for this endpoint because of the following:

5. The 28-day rat study (oral) with CAS No. 67124-09-8 found no NOAEL (liver effects) at doses ranging from 100 to 1000 mg/kg. The 90-day rat study (dermal) with CAS No. 68511-50-2 found systemic effects (increase in liver/body weight ratios, spleen size, various hematological parameters) at *dermally applied* doses of 250 mg/kg or higher; although a NOAEL for some hematological effects was as low as 50 mg/kg. Although there are exceptions, it is generally believed that a test substance administered via the oral route is more bioavailable (absorbed into systemic circulation) than via the dermal route. The data suggest either that 68511-50-2 is more toxic than 67124-09-8 or that the dermal route is more toxic than the oral route for these compounds.

6. CAS No. 68515-88-8 was tested in a 28-day inhalation study with rats, followed by a three-week recovery period. Results showed a variety of effects that were not reversible at the end of the recovery period - decrease in body weight gain and increase in organ weights (spleen, adrenal, testes, lung, and heart). The dose level for these effects was 0.15 mg/L (150 mg/m^3), which is difficult to equate to the oral and dermal doses used in the two studies referenced in 1. above.

7. CAS No. 68515-88-8 appears to be more severely irritating to the skin of rats than CAS No. 68511-50-2. There is no dermal repeat dose study with CAS No. 67124-09-8.

8. Finally, none of the repeat dose studies were performed with either of the proposed category members that are derived from linear olefins and also have cyclic components (CAS No. 72162-15-3 or 67762-55-4).

Genotoxicity Studies. Nine different genotoxicity robust summaries were submitted on five different chemicals (four of the five alkyl sulfide proposed alkyl sulfide category members–CAS No. 72162-15-3 being the only one not tested) and one chemical (CAS No. 91770-97-4) used as an analogue for CAS No. 67762-55-4; the former is a C12-C16 sulfide and the latter is a C15-C18 sulfide). Although some of the summaries were missing important information, these omissions did not change EPA's weight-of-evidence analysis that all the tested materials were negative for genotoxicity in the systems tested. In fact, EPA considered the summary of the *in vivo* micronucleus test with the analog chemical inadequate for the purposes of the US HPV program. EPA has the following comments on the robust summaries (the comments reflect the information presented in the robust summaries; information in the full study report may address some of the issues identified):

CAS No. 67124-09-8

Reverse mutation in Salmonella typhimurium and Escherichia coli - There was no indication of the number of plates used per concentration tested.

CAS No. 68511-50-2

In vivo micronucleus (as an add-on to a 13-week dermal subchronic toxicity study) - There was no positive control.

Reverse Mutation in Salmonella - The highest concentration tested, 1.0 µl/plate, is low but was probably reasonable when the test was performed (1978). There is no indication of the following in the summary: (1) toxicity at the highest concentration tested, (2) the number of plates used per concentration tested, and (3) how the number of revertants were counted, e.g., automatic colony counter, by hand, etc.

CAS No. 68515-88-8

Reverse mutation in Salmonella - There is no indication of: (1) how test concentrations were chosen, (2) toxicity at highest concentration tested, and (3) how mutant colonies were counted. In the absence of any sign of toxicity the highest concentration tested may be too low.

In vivo micronucleus -The abbreviation NME for normochromatic erythrocyte, rather than NCE, is unusual. There is no indication: (1) of the tissue from which blood was collected for preparation of PCEs and NCEs, (2) of the method used to prepare slides for scoring, and (3) that slides were coded before analysis.

CAS No. 67762-55-4

Reverse mutation in Salmonella and Escherichia coli - There is no indication of: (1) how revertant colonies were counted, (2) how toxicity was judged, and (3) what the positive control cultures were.

CAS No. 91770-97-4 (as an analogue for CAS No. 67762-55-4)

In vivo micronucleus -The assay was done with 5 male mice per dose. Both the OECD and EPA guidelines in effect at the time (1996)and which the summary says were followed call for 10 animals, 5 males and 5 females, per dose group. The dose for the positive control is not stated. The rationale for eliminating statistical analysis is unacceptable; statistics should have been performed to determine if the number of PCE in the treatment group was significantly lower than that in the vehicle control. Treatment regimen, i.e., dosing over 3 days, for the test chemical should be justified. It is not clear how cytotoxicity was determined; it seems to be based upon a reduction in PCE over that seen in the controls. Unless this is based upon PCE:NCE ratio EPA does not understand its use here. There is no indication of : (1) PCE:NCE ratio or (2) how PCE were counted; e.g. flow cytometry, fluorescence microscopy. It states that the positive control behaved appropriately but the numbers of PCE are not stated for either the positive or negative controls.

All nine studies submitted were negative for genotoxicity in a variety of test systems. EPA believes that, for the genotoxicity endpoint, the information is adequate for a category/read-across approach for evaluating CAS No. 72162-15-3.

Reproductive Toxicity Studies. No reproductive toxicity data considered adequate under the HPV Program for the alkyl sulfide category are available.

Developmental Toxicity Studies. No developmental toxicity data considered adequate under the HPV Program for the alkyl sulfide category are available.

Ecotoxicity Studies.

CAS No. 68511-50-2.

All four ecotoxicity studies supplied for these endpoints were for this chemical and were considered inadequate. The comments below reflect the information presented in the robust summaries; information in the full study report may address some of the issues identified.

Fish Acute Toxicity. Two 96-h fish acute robust summaries submitted for using TSCA test guideline #797.1400, and the OECD guideline #203 for the WAF and WSF static renewal tests, respectively, were done with nominal concentrations. The Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD, June 2000 - available on the OECD Web site at http://www.oecd.org/ehs/test/monos.htm) states that the LC50, EC50, and NOEC concentrations from these tests should be calculated from the measured concentrations of test substance and tested within the test substance water solubility range. It appears this procedure was not followed during these tests. In addition, the summary did not specify if efforts were made to measure the water saturation concentrations for these chemicals or if a preliminary experiment was done demonstrating that the test substance preparation regime is sufficient to maximize the concentration. In addition, the summaries did not mention whether a vehicle was used to solubilize the test substance.

Daphnid acute toxicity. Information on the vehicle, if any, used to solubilize the test substance was not reported. Test concentration was not reported. The 48-h daphnid acute toxicity test is too short in

duration to allow this chemical to reach equilibrium with the test organisms. The test was not conducted in the manner prescribed for hydrophobic substances by the difficult substances and mixtures guidance (see comments on review of fish acute tests).

Algal Toxicity. The 96-h algal EC50 toxicity was tested above the water solubility limit. Test concentrations were not measured at the start and at the end of the test. The test was not conducted in a manner prescribed by the difficult substances and mixtures guidance with regard to proper test substance preparation.

FOLLOWUP ACTIVITY

EPA requests that the Sponsor advise the Agency within 60 days how it intends to pursue activities on the chemicals in its submission.